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## RESEARCH ARTICLE

# Urine is an important nitrogen source for plants irrespective of vegetation composition in an Arctic tundra: Insights from a $^{15}\text{N}$ -enriched urea tracer experiment

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**Abstract**

1. Mammalian herbivores can strongly influence nitrogen (N) cycling and herbivore urine could be a central component of the N cycle in grazed ecosystems. Despite its potential role for ecosystem productivity and functioning, the fate of N derived from urine has rarely been investigated in grazed ecosystems.
2. This study explored the fate of  $^{15}\text{N}$ -enriched urea in tundra sites that have been either lightly or intensively grazed by reindeer for more than 50 years. We followed the fate of the  $^{15}\text{N}$  applied to the plant canopy, at 2 weeks and 1 year after tracer addition, in the different ecosystem N pools.
3.  $^{15}\text{N}$ -urea was rapidly incorporated in cryptogams and in above-ground parts of vascular plants, while the soil microbial pool and plant roots sequestered only a marginal proportion. Furthermore, the litter layer constituted a large sink for the  $^{15}\text{N}$ -urea, at least in the short term, indicating a high biological activity in the litter layer and high immobilization in the first phases of organic matter decomposition.
4. Mosses and lichens still constituted the largest sink for the  $^{15}\text{N}$ -urea 1 year after tracer addition at both levels of grazing intensity demonstrating their large ability to capture and retain N from urine. Despite large fundamental differences in their traits, deciduous and evergreen shrubs were just as efficient as graminoids in taking up the  $^{15}\text{N}$ -urea. The total recovery of  $^{15}\text{N}$ -urea was lower in the intensively grazed sites, suggesting that reindeer reduce ecosystem N retention.
5. *Synthesis.* The rapid incorporation of the applied  $^{15}\text{N}$ -urea indicates that arctic plants can take advantage of a pulse of incoming N from urine. In addition,  $\delta^{15}\text{N}$  values of all taxa in the heavily grazed sites converged towards the  $\delta^{15}\text{N}$  values for urine, bringing further evidence that urine is an important N source for plants in grazed tundra ecosystems.

**KEYWORDS**

above- and belowground linkages, Arctic tundra, cryptogams, grazing intensity, microbial N biomass, N labelling, nutrient cycling, plant–herbivore interactions, plant nutrient uptake, urine

## 1 | INTRODUCTION

Herbivores play a key role in terrestrial ecosystem by modifying plant and microbial community composition, nutrient cycling and productivity (Bardgett & Wardle, 2003; Frank & Groffman, 1998; McNaughton, 1979). Herbivores influence ecosystem structure and functioning via three main mechanisms: plant defoliation, trampling, and nutrient return in the form of dung and urine (Mikola et al., 2009; Olofsson, 2009). By providing highly decomposable resources rich in labile nutrients, and by stimulating soil microbial activities and thus promoting carbon (C) and nitrogen (N) mineralization, the input of dung and urine can enhance soil nutrient availability and promote plant nutrient acquisition and growth (Bardgett, Keiller, Cook, & Gilburn, 1998; Frank, Inouye, Huntly, Minshall, & Anderson, 1994; Hobbs, 1996; Mikola et al., 2009; Olofsson, 2009; Ruess & McNaughton, 1987; Stark & Kytöviita, 2006). Via enhanced plant productivity, the deposition of dung and urine may at certain conditions create a positive feedback that further enhances the intensity of herbivory (McNaughton, 1979).

Although the relative importance of urine and dung may vary depending on forage quality and herbivore type, it has been estimated that half of the N will be often excreted as urine and the other half as dung (Hobbs, 1996; Mosbacher, Kristensen, Michelsen, Stelvig, & Schmidt, 2016). However, urine and dung differ greatly in their chemical composition (Floate, 1970; Hobbs, 1996). While dung is composed of a mixture of non-assimilated and undigested plant materials, urine is composed of a large array of assimilated N, mainly urea ( $\text{CO}(\text{NH}_2)_2$ ). Urea can either be taken up by the plant either directly or after rapid hydrolyzation into soluble ammonium (Witte, 2011), and is thus more readily available to plants than most N compounds in dung (Floate, 1970; Ruess & McNaughton, 1987; Seagle, McNaughton, & Ruess, 1992).

There are only few studies investigating the effect of dung on natural ecosystems (Barthelemy, Stark, & Olofsson, 2015; Mikola et al., 2009; Ruess, Hik, & Jefferies, 1989; van der Wal, Bardgett, Harrison, & Stien, 2004), and the return of nutrients in form of urea has attracted even less attention outside agricultural fields, despite that is a fairly large N source for plants in many systems. Studying the effect of N compounds derived from natural urine deposition is highly complex due to methodological challenges in tracing and quantifying urine in natural ecosystems. The use of  $^{15}\text{N}$ -enriched urea might be a way to overcome some of these challenges since the application of  $^{15}\text{N}$ -enriched compounds is a powerful technique to follow a specific element through a system without altering its natural behaviour (Dawson, Mambelli, Plamboeck, Templer, & Tu, 2002) and is widely used to study N cycling and N uptake in a large variety of ecosystems (Clemmensen, Sorensen, Michelsen, Jonasson, & Strom, 2008; Frank, Evans, & Tracy, 2004; Martinsen, Austrheim, Myserud, & Mulder, 2011; Mulholland et al., 2000).

The N deposition to the soil in the form of urine and dung might have special importance in the Arctic where decomposition processes and mineralization of nutrients are low (Van Cleve & Alexander, 1981; Weintraub & Schimel, 2003), and nutrient availability is commonly

seen as the major factor limiting plant growth (Aerts & Chapin, 2000; Schimel & Bennett, 2004). Most of the soil N is bound in complex organic compounds and large proportions of the ecosystem nutrient pool can be immobilized by soil micro-organisms which can act as strong competitors with plants for the available nutrients (Jonasson, Michelsen, Schmidt, & Nielsen, 1999; Jonasson, Michelsen, Schmidt, Nielsen, & Callaghan, 1996; Michelsen et al., 1999; Schimel & Chapin, 1996).

Reindeer (*Rangifer tarandus*) is a keystone herbivore in arctic ecosystems (Bernes, Bråthen, Forbes, Speed, & Moen, 2015) and reindeer dung and urine could thus be an important supply of nutrients in these ecosystems. However, the two existing dung manipulation studies revealed that the return of nutrients to the soil is slow, and it can take several years before plant growth is stimulated (Barthelemy et al., 2015; van der Wal et al., 2004), especially in dry heath habitats where low moisture content retard reindeer pellets decay (Skarin, 2008). The importance of herbivores on plant nutrition might be much larger than estimated in these studies since urine was not considered. In our N-limited systems (Sitters, te Beest, Cherif, Giesler, & Olofsson, 2017), a readily available N source like urine could be expected to be rapidly taken up by vascular plants and thus favour plant growth and the production of forage plants for the reindeer. However, a large proportion of N from the urine could be also immobilized by mosses and soil microbes. Mosses, which are abundant in many arctic ecosystems, are very efficient in capturing and storing nutrients (Jónsdóttir, Callaghan, & Lee, 1995; Weber & Van Cleve, 1981) and could thus prevent the N from urine to reach the soil and to be available for plant root uptake (van der Wal & Brooker, 2004; van der Wal et al., 2003). On the other hand, even after reaching the soil, a large fraction of labile nutrients is often immobilized by microbes rather than being directly available for plant growth (Nordin, Schmidt, & Shaver, 2004).

The aim of this study was to investigate how N added in the form of urea is taken up and retained by the various compartments of grazed ecosystems. The tracer study was conducted along a 50-year-old reindeer pasture fence, providing us a unique system to compare the fate of  $^{15}\text{N}$ -urea in two sites with highly contrasting grazing intensities, a lightly and heavily grazed tundra. Intense defoliation, trampling and dung and urine deposition have transformed the initial nutrient-poor heath vegetation dominated by mosses and dwarf shrubs into a nutrient-rich and graminoid-dominated vegetation where the abundance of mosses is severely reduced and dwarf shrubs have almost disappeared (Olofsson, Stark, & Oksanen, 2004). Urine deposition is drastically higher in the heavily grazed tundra since approximately  $0.3 \text{ g N m}^{-2} \text{ year}^{-1}$  is added as urine in the heavily grazed system, and less than  $0.01 \text{ g N m}^{-2} \text{ year}^{-1}$  in the lightly grazed system (te Beest, Sitters, Menard, & Olofsson, 2016), if we assume half of the N is excreted as urine and half as dung (Hobbs, 1996; Mosbacher et al., 2016). Vascular plants in grazed systems should thus be adapted to efficiently utilize N from urine, but the same may not be true for lightly grazed systems.

To study how N from urine is taken up by different ecosystem compartments in the two grazing systems, we sprayed the isotopic labelled urea over the vegetation and we measured the recovery

of the  $^{15}\text{N}$ -enriched urea in the vegetation, soil and microbial N pools 2 weeks and 1 year after tracer addition. We hypothesized that: (1) Urine is a more important N source for vascular plants in heavily grazed sites than in lightly grazed sites since not only urine deposition is higher but also a larger fraction of N from urine is taken up by vascular plants and a smaller fraction is immobilized by the moss layer and soil microbes; (2) Graminoids are more efficient in taking up N from urine than other functional groups of vascular plants.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

The research site is located above the tree line (600–700 m a.s.l.) on the northern slope of Raisduoddar Fjell (69°31'N, 21°19'E) in the suboceanic northern Norway. Reindeer husbandry forms the dominant land-use in the area. At the study site, the annual precipitation is 935 mm and the annual mean temperature is  $-0.6^{\circ}\text{C}$  (2006–2015, Norwegian Water Resources and Energy Directorate, [www.se-norge.no](http://www.se-norge.no)). The snow-free plant growing season is typically less than 3 months, extending from mid-June to mid-September.

The study was conducted across a reindeer pasture fence established in the 1960s to reduce the risk that reindeer enter migration areas during the summer. The fence runs for several kilometres across the tundra and separates the summer range from the spring and autumn migration range. Reindeer are mainly present in the site in the second half of August, and there is no difference in the timing of the reindeer grazing between the grazing regimes. The sites in the migration range are almost ungrazed with only little trampling, dung deposition and defoliation (Olofsson et al., 2004) and are thus referred to as the lightly grazed sites. These sites are dominated by dwarf shrub heath vegetation with typical species such as *Empetrum hermaphroditum*, *Vaccinium vitis-idaea*, *Vaccinium uliginosum*, *Vaccinium myrtillus* and *Betula nana*. Mosses and lichens are abundant with as the most common species *Pleurozium schreberi*, *Polytrichum* sp. and *Dicranum* sp., and *Nephroma arctica*, *Peltigera* sp. and *Cladonia* sp. respectively. Graminoids and herbs are present, but rare (Olofsson, Kitti, Rautiainen, Stark, & Oksanen, 2001).

The sites in the summer range, hereafter referred to as heavily grazed sites, are intensively utilized by reindeer, resulting in intense defoliation, dung deposition and trampling. The original moss and dwarf shrub heath vegetation has been transformed into a graminoid-dominated heath vegetation with strong increase in sedges in particular *Carex bigelowii*, *Carex lachenalii* and *Festuca ovina*, and grasses like *Deschampsia flexuosa*, *Deschampsia cespitosa*, *Poa alpina* and *Phleum alpinum* with only scattered dwarf shrubs (Olofsson et al., 2001). Soil N concentrations and microbial respiration are generally much lower in the lightly than in the heavily grazed area (Stark, Strommer, & Tuomi, 2002; Stark & Väisänen, 2014). Trampling indicators and dung counts reveal that reindeer density is about 100 times higher in the heavily grazed than in the lightly grazed sites (Olofsson et al., 2004).

### 2.2 | Experimental design

At the peak of the growing season, in late July 2011, we established 10 blocks along the reindeer fence, with two plots (1 m × 1 m) at each side of the fence within each block (in total 40 plots). All plots were placed within a distance of 5 m from the fence, and selected to have as similar abiotic conditions as possible. We assumed that the main differences in vegetation between the sides of the fence are only controlled by a top-down effect of reindeer grazing. In each block, plots were randomly allocated to control or  $^{15}\text{N}$ -enriched urea addition plots.

The  $^{15}\text{N}$ -labelled urea (Urea- $^{15}\text{N}$ , 98 atom %  $^{15}\text{N}$ , Sigma-Aldrich), diluted in 2 L of stream water, was sprayed uniformly over the vegetation at a rate of 0.1 g N/m<sup>2</sup>, on 29 July 2011. During the tracer application, the  $^{15}\text{N}$  addition plots were isolated from the surrounding vegetation by a 50-cm-high plastic film placed around them. Applying this trace amount of urea, which is  $^{15}\text{N}$ -enriched relative to any occurring natural level, allowed us to trace the N-urea in the different ecosystem N pools but precluded fertilization effect. The N added represented about 50% of the annual atmospheric N deposition at the site (0.2 g m<sup>-2</sup> year<sup>-1</sup>, [www.environment.no](http://www.environment.no)). Following each tracer addition, control plots received the equivalent amount of non- $^{15}\text{N}$ -enriched water. To mimic natural urine deposition in a grazed system, the  $^{15}\text{N}$  addition plots did not receive any additional stream water to wash off the vegetation from the added  $^{15}\text{N}$ -urea. It rained heavily during the period between the tracer addition and the first harvest (H. Barthelemy, pers. obs.) and since differences in plant  $^{15}\text{N}$  enrichment between the 2 weeks and 1 year harvest was small, we are confident that most of the  $^{15}\text{N}$  have been assimilated by the plants prior the first harvest.

### 2.3 | Sampling

Above-ground and below-ground part of plants and soil samples were collected about 10 days after the application of the tracer (10–14 August 2011) in all the 40 plots, and in all  $^{15}\text{N}$  addition plots and three randomly chosen control plots in each grazing treatment after 1 year (11–13 August 2012). All above-ground vegetation was harvested in a 25 cm × 25 cm subplot and was sorted at the species level, except from graminoids and cryptogams that were treated collectively. The above-ground biomass was then divided into the following seven compartments; (1) *B. nana*, (2) Other deciduous shrub species, (3) *E. hermaphroditum*, (4) Other evergreen shrub species, (5) Forbs, (6) Graminoids and (7) Cryptogams. The cryptogams include bryophytes and lichens. The complete species composition and abundance for the compartments Other deciduous shrubs, Other evergreen shrubs, Forbs and Cryptogams are presented in Tables S1 and S2. The litter layer was collected in the same subplots and included both fresh litter and older plant remains.

Root biomass was sampled from soil cores (area 44 cm<sup>2</sup>) in the middle of each subplot at a depth of 5 cm corresponding to the active root zone at the site. The sampled roots, not sorted at the species level, were thoroughly washed to remove organic matter residue and

mineral components. All the plant samples were oven-dried (60°C, 48 hr) and weighed. To estimate the soil extractable and microbial N pools, three soil cores of an estimated volume of 500 cm<sup>3</sup> were taken at each plant sampling sites into the top soil (horizon A). The soil samples were kept frozen until analysis.

## 2.4 | Chemical analysis

Plant samples were finely ground (Retsch® MM301 ball mill, Haan, Germany) and subsamples of this plant material were encapsulated into preweighted tin capsules. The  $\delta^{15}\text{N}$  and elemental N concentration was measured on an Isoprime isotope ratio mass spectrometer (Isoprime Ltd., Cheadle Hulme, UK) coupled to a CN elemental analyser (Eurovector, Milan, Italy), at the Stable Isotope Facility at Department of Biology, University of Copenhagen.

Soil samples were thawed at room temperature and live plant materials, undecomposed dead plant parts and roots were removed by sieving (mesh 2 mm). The soil water content was measured gravimetrically (105°C, 12 hr) and organic matter content was determined by loss-on-ignition (475°C, 12 hr). Soil microbial biomass N was determined as the chloroform labile fraction using the chloroform fumigation-extraction techniques described by (Brookes, Landman, Pruden, & Jenkinson, 1985). A subsample of 2 g of soil was chloroform fumigated (18 hr) in order to release the nutrients contained in the microbial biomass and extracted with 50 ml of 0.5 M K<sub>2</sub>SO<sub>4</sub>. Another 2 g of subsample was extracted without the fumigation for analysing the total extractable N in the soil solution. All the extracts were filtered through Whatman No 1 filter paper. Total extractable N in the extracts was determined by oxidizing the entire extractable N to NO<sub>3</sub><sup>-</sup> (Williams et al., 1995) and then analysed it by automated flow injection analyser (N, FIA Perstorp). The microbial N content (N<sub>mic</sub>) was calculated by subtracting the total extractable N in the unfumigated extracts (N<sub>e</sub>) from the N present in the fumigated extracts (N<sub>f</sub>), using the microbial correction factor K<sub>EN</sub> = 0.4 representative for organic soils, to account for microbial tissue N that is not released by exposure to chloroform (Jonasson et al., 1996).

To determine extractable  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N}_e$ ) and microbial  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N}_{\text{mic}}$ ), fumigated and unfumigated extracts were analysed for <sup>15</sup>N/<sup>14</sup>N isotopic ratio following a modification of the acid trap diffusion technique on a subsample of 10 ml of digested extracts (Holmes, McClelland, Sigman, Fry, & Peterson, 1998). The methodological considerations of analysing  $\delta^{15}\text{N}_e$  and  $\delta^{15}\text{N}_{\text{mic}}$  using chloroform fumigation are discussed in detail by Dijkstra et al. (2006). To reduce NO<sub>3</sub><sup>-</sup>-N to NH<sub>4</sub><sup>+</sup>-N and to volatilize NH<sub>4</sub><sup>+</sup>-N to NH<sub>3</sub>, 0.4 g of Devarda's alloy and 0.2 g of magnesium oxide were added to digested extracts. Vials were immediately sealed with a cap suspending an acidified (10 ml of 1.5 M KHSO<sub>4</sub>) glass microfiber filter discs (1.4 cm diameter; Whatman GF/A; GE Healthcare Life Sciences, Chicago, IL, USA) and incubated at 22°C for 7 days to trap all the volatilized NH<sub>3</sub> onto the acidified discs. The total amount of N in the diffusion traps confirmed that the diffusion was complete. Diffusion discs were then dried in a desiccator, encapsulated into preweighted tin capsules and analysed using a PDZ

Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, University of California, Davis.

## 2.5 | Isotopic calculation

The natural abundance of <sup>15</sup>N was expressed as

$$\delta^{15}\text{N}(\text{‰}) = 1000 \times \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right)$$

where  $R_{\text{sample}}$  is the isotope ratio of the sample (<sup>15</sup>N/<sup>14</sup>N) relative to a standard calibrated against atmospheric N<sub>2</sub>. The isotope signature of the microbial biomass N pool  $\delta^{15}\text{N}_{\text{mic}}$  was determined using isotope mass balance as

$$\delta^{15}\text{N}_{\text{mic}}(\text{‰}) = \frac{\delta^{15}\text{N}_f \times N_f - \delta^{15}\text{N}_e \times N_e}{N_{\text{mic}}}$$

where  $\delta^{15}\text{N}_f$  and  $\delta^{15}\text{N}_e$  are the  $\delta^{15}\text{N}$  measured in chloroform fumigated (microbial + extractable N) and extractable (unfumigated) samples, the  $N_f$  and  $N_e$  are the total extractable N (mg N/kg SOM) determined for the fumigated and unfumigated samples, respectively, and  $N_{\text{mic}}$  is the extracted microbial N. The urea-<sup>15</sup>N enrichment in each ecosystem N pools was calculated as the <sup>15</sup>N atomic frequency in excess as

$$\text{Atom } ^{15}\text{N} \% \text{ excess} = \text{Atom } \% \text{ tracer} - \text{Atom } \% \text{ background}$$

where Atom % tracer is the <sup>15</sup>N atomic frequency of a sample collected in the <sup>15</sup>N-urea addition plot and the Atom % background is the <sup>15</sup>N atomic frequency of the corresponding sample collected in the non-labelled plot.

The percentage tracer recovery in each ecosystem N pool was estimated as

$$\% \text{tracer recovery } ^{15}\text{N} = \frac{(\text{Atom } ^{15}\text{N excess} \times N \times \text{Mass}) / 10^4}{\text{Total amount of tracer added}} \times 100$$

where Atom <sup>15</sup>N excess is the urea-<sup>15</sup>N enrichment (%), N is the N content (%), Mass is the total mass of each pool biomass or soil (g m<sup>-2</sup>) and the total amount of the tracer added corresponds to the 0.1 g m<sup>-2</sup> of the isotopic labelled urea. Tracer recovery was corrected for the fractional abundance of <sup>15</sup>N in the tracer (98 Atom % <sup>15</sup>N). We assumed negligible N isotope fractionation during tracer movement in the different ecosystem pools (Robinson, 2001) and no difference in <sup>15</sup>N natural abundance within the pools from 2011 and 2012. We cannot exclude minor tracer losses at the plot borders or on the plastic protection film at the application. The <sup>15</sup>N recoveries reported here in this study could thus be an underestimate of the true <sup>15</sup>N recoveries within the system.

## 2.6 | Statistics

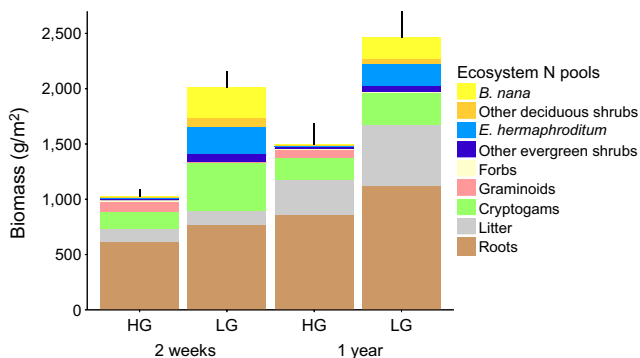
All statistical analyses were performed using the statistical package R (R development Core Team, 2016). Plant biomass, N pools, tracer recovery and the calculated <sup>15</sup>N atom% excess were tested using linear mixed-effects models with grazing intensity and time following tracer

addition as fixed factors and Plot as a random effect. This analysis avoids temporal pseudoreplication by taking into account the two recordings of the same plots at 2 weeks and 1 year after tracer addition. Linear contrasts were used to examine the differences among grazing intensities across time. Prior to statistical analysis, *B. nana*, Other deciduous shrubs, *E. hermaphroditum* and Other evergreen shrubs pools were merged into the single group Shrubs. All variables were ln transformed, except for the tracer recovery that was arcsine transformed, to fulfil the assumptions of normality and homoscedasticity. Differences in plant  $\delta^{15}\text{N}$  natural abundance among and within the ecosystem N pools in response to grazing were tested using a two-way ANOVA with ecosystem N pools and grazing intensity as categorical variables. A Tukey's studentized range honestly significant difference test was used to examine posteriori differences among ecosystem N pools' means. *Betula nana* was only found on the lightly grazed control plots, thus, the effect of grazing intensity on  $\delta^{15}\text{N}$  natural abundance could not have been tested for this plant species. Akaike's information criteria and residual plots were used to assess the fit of the models.

### 3 | RESULTS

#### 3.1 | Plant biomass

The total biomass of plants and litter was higher in lightly grazed sites than in the heavily grazed sites, and was also higher in 2012 than in 2011 across all sites (Figure 1, Table 1). Especially the litter pool and root biomass was larger in 2012 (Figure 1, Table 1). The cryptogam compartment consists predominantly of bryophytes, since lichens were rare (Tables S1 and S2). As expected, reindeer strongly influenced the plant community composition (Tables S1 and S2). Graminoid biomass was more than 20 times higher in the heavily grazed sites than in the lightly grazed sites (Figure 1, Table 1). Forb biomass was also higher in the heavily grazed sites,



**FIGURE 1** Above-ground and below-ground plant biomass, and plant litter ( $\text{g/m}^2$ ) in each  $^{15}\text{N}$ -enriched plots at the heavily (HG) and lightly grazed (LG) sites at 2 weeks and 1 year after the addition of the labelled  $^{15}\text{N}$ -urea within the system. Bars represent mean biomass for each ecosystem N pools and the error bars account for the standard error for the total biomass for each sites in each sampling year [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

but the difference was much smaller (Figure 1, Table 1). Total shrub and cryptogam biomass was on the other hand 6% and 50% of the biomass in the heavily grazed sites respectively (Figure 1). There was finally more litter in the lightly than in the heavily grazed sites (Figure 1, Table 1). Exact values for plant biomass, N pool sizes, recovery and  $^{15}\text{N}$  enrichment in the different ecosystem N pools are shown in Tables S3 and S4.

#### 3.2 | N pool sizes

The total N pool did not differ between the two grazing regimes (Figure 2, Table 1). Litter, roots, soil microbes and soil extractable N were all large N pools in both grazing regimes, and contained collectively between 70% and 80% of the whole N pool (Figure 2). There was twice as much extractable N in the heavily grazed soils compared to the lightly grazed soils (Figure 2, Table 1). The microbial N pool size did not differ between the two grazing regimes. Cryptogams contained between 3% and 5% of the total N pool in both grazing treatments, so the larger cryptogam biomass in the lightly grazed sites did not transfer into the N pool (Figure 2, Table 1). The shift in the dominating vegetation from shrubs to graminoids resulted in correspondingly smaller N pools in shrubs and larger N pools in graminoids in the heavily grazed sites (Figure 2, Table 1).

#### 3.3 | Initial recovery and $^{15}\text{N}$ enrichment

The total recovery of N from urea after 2 weeks was higher in the lightly grazed sites than in the heavily grazed sites (Figure 3, Table 1). Cryptogams were the largest sink of the added  $^{15}\text{N}$  in both grazing intensities, but the pool was twice as large in the lightly grazed sites (Figure 3, Table 1). The litter layer and graminoids were also heavily  $^{15}\text{N}$  enriched in both grazing regimes and were, after cryptogams, the largest N sinks in the heavily grazed sites. In the lightly grazed sites, shrubs replaced the graminoids as the second largest N sink (Figure 3). Roots were slightly more  $^{15}\text{N}$  enriched in the heavily grazed sites than in the lightly grazed sites, although both were really weak sinks of N from urea (Figures 3 and 4, Table 1). Soil extractable N pool was also a weak N sink in both systems. Shrubs were more  $^{15}\text{N}$  enriched in lightly grazed sites than in heavily grazed sites (Figure 4, Table 1).  $^{15}\text{N}$  enrichment did not differ between the deciduous and evergreen shrubs (Figure S5).

#### 3.4 | Recovery and $^{15}\text{N}$ enrichment after 1 year

Twenty-five percent (heavily grazed) and 40% (lightly grazed) of the initially recovered N was lost after 1 year (Figure 3), but the difference was not statistically significant (Table 1). The N recovery and  $^{15}\text{N}$  enrichment decreased in shrubs, graminoids and forbs (Figures 3 and 4, Table 1). N recovery decreased also in cryptogams and  $^{15}\text{N}$  enrichment decreased also in litter (Figures 3 and 4, Table 1). In contrast, the  $^{15}\text{N}$  recovery in roots was higher after 1 year (Figure 3, Table 1). While the  $^{15}\text{N}$  recovery in shrubs remained higher in the lightly grazed sites and  $^{15}\text{N}$  recovery in graminoids remained higher



	Biomass	N pool	Recovery	<sup>15</sup> N enrichment
Total				
Grazing intensity	46.79***	0.80	14.46**	–
Time	8.01*	86.90***	19.401***	–
Grazing intensity: Time	0.55	1.40	3.72	–
Shrubs				
Grazing intensity	48.23***	164.2***	150.00***	90.63***
Time	2.843	1.491	35.60***	11.57**
Grazing intensity: Time	0.50	10.99**	24.13***	9.12**
Forbs				
Grazing intensity	9.84**	2.85	3.59	2.24
Time	0.10	0.03	5.93*	20.25***
Grazing intensity: Time	0.99	1.09	5.79*	1.62
Graminoids				
Grazing intensity	121.70***	120.90***	70.82***	0.01
Time	2.165	2.81	21.8***	68.69***
Grazing intensity: Time	8.03*	0.14	22.11***	0.604
Cryptogams				
Grazing intensity	5.91**	1.13	4.49*	2.38
Time	1.74	1.44	22.88***	1.46
Grazing intensity: Time	2.50	3.89	4.76*	2.24
Litter				
Grazing intensity	5.381*	0.057	0.03	0.48
Time	48.30***	74.97***	1.32	49.72***
Grazing intensity: Time	0.33	1.37	2.48	1.51
Roots				
Grazing intensity	3.11	2.50	18.0***	11.75**
Time	4.08	8.20*	5.42*	1.76
Grazing intensity: Time	0.27	0.11	0.07	0.01
Microbial N				
Grazing intensity	–	0.04	0.06	0.60
Time	–	0.11	1.21	2.29
Grazing intensity: Time	–	1.40	0.41	0.65
Soil extractable N				
Grazing intensity	–	15.34**	5.57*	2.26
Time	–	7.45*	3.53	3.38
Grazing intensity: Time	–	0.01	0.34	0.01

F-values are presented ( $df = 1,18$ ) and the asterisks denote significance at \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ . “–” indicates no value.

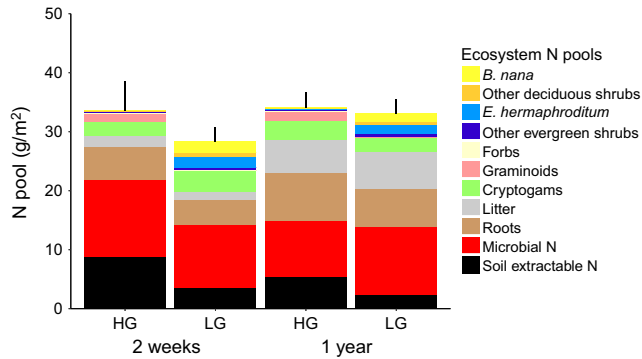
in the heavily grazed (Figure 3, Table 1), the recovery in cryptogams did not differ between grazing regimes any longer after 1 year (Figure 3).

### 3.5 | Plant <sup>15</sup>N natural abundance

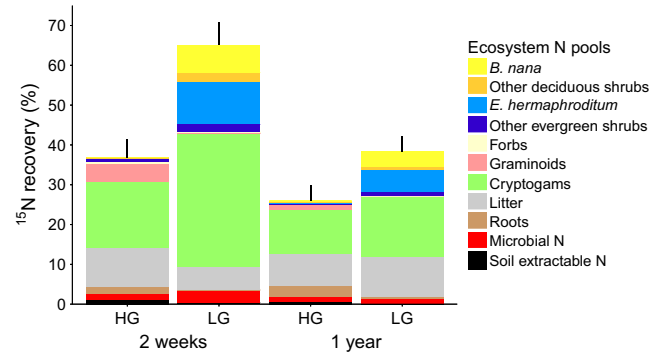
Plant <sup>15</sup>N natural abundance differed between grazing regimes and plant N pools with a range from –11.2‰ to 7.7‰ (Figure 5, Table 2). The <sup>δ</sup><sup>15</sup>N values were lowest in evergreen shrubs, followed by

**TABLE 1** Analyses of variance on the effects of grazing intensity and time after the tracer addition on biomass (g/m<sup>2</sup>), N pool (g/m<sup>2</sup>), tracer recovery (%) and <sup>15</sup>N enrichment (Atom % <sup>15</sup>N excess). Grazing intensity corresponds to the lightly and heavily reindeer grazed sites and Time corresponds to the sampling dates after the tracer addition (2 weeks and 1 year)

deciduous shrubs and cryptogams, while graminoids had the highest <sup>δ</sup><sup>15</sup>N values (Figure 5). The variance in <sup>δ</sup><sup>15</sup>N values among plant functional groups was lower ( $p < .001$ ) in the heavily grazed sites (1.9‰) than in the lightly grazed sites (8.5‰). The <sup>δ</sup><sup>15</sup>N were about 3‰ higher in heavily grazed sites compared to lightly grazed sites in *E. hermaphroditum*, other evergreen shrubs and other deciduous shrubs ( $p < .001$ ); while it was 2.4‰ lower in graminoids in the heavily grazed sites ( $p = .021$ ). The litter <sup>δ</sup><sup>15</sup>N was higher by 3.3‰ in the heavily grazed sites (Figure 5).

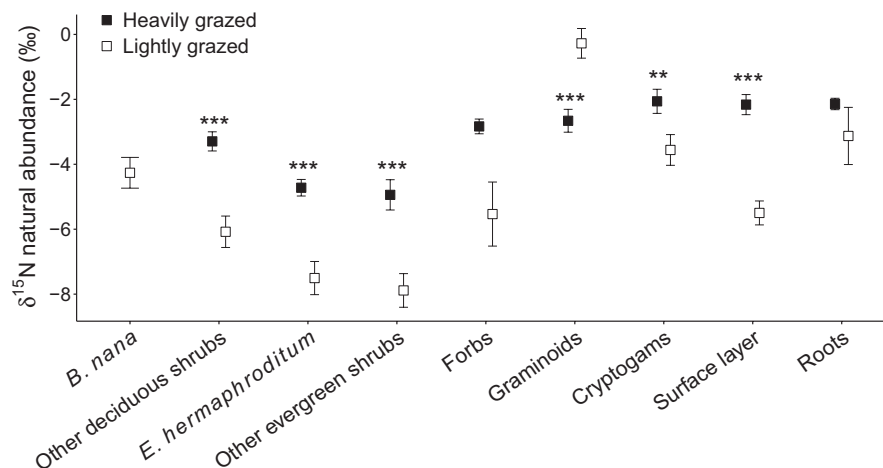
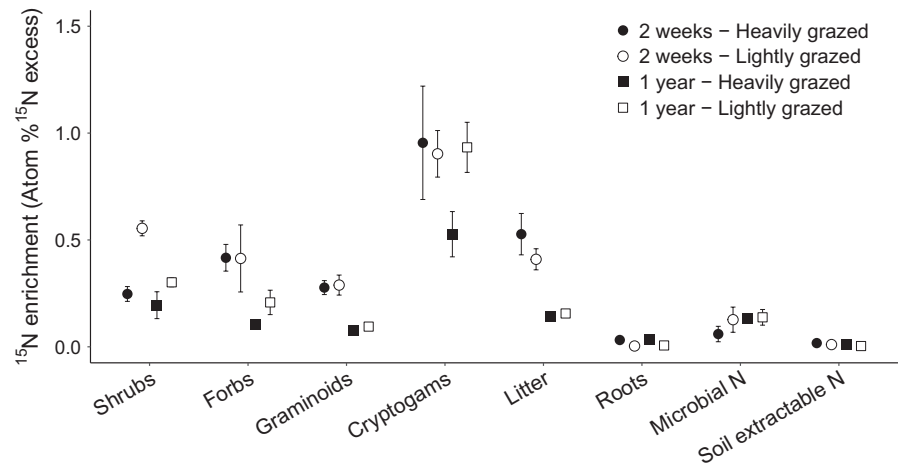


**FIGURE 2** N pool ( $\text{g/m}^2$ ) at the heavily (HG) and lightly grazed (LG) sites at 2 weeks and 1 year after the addition of the labelled  $^{15}\text{N}$ -urea within the system. Bars represent means for each ecosystem N pools and the error bars account for the standard error of the total N pool in each sites for each sampling year [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 3**  $^{15}\text{N}$  recovery (%) at the heavily (HG) and lightly grazed (LG) sites at 2 weeks and 1 year after the addition of the labelled  $^{15}\text{N}$ -urea within the system. Bars represent means for each ecosystem N pools and the error bars account for the standard error of the total recovery in each sites for each sampling year [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**FIGURE 4**  $^{15}\text{N}$  enrichment (Atom %  $^{15}\text{N}$  excess) at the heavily and lightly grazed sites at 2 weeks and 1 year after the addition of the labelled  $^{15}\text{N}$ -urea within the system. Mean values  $\pm$  standard errors are presented. In cases where the standard errors are not visible, they are smaller than the data point



**FIGURE 5**  $^{15}\text{N}$  natural abundance (‰) for the above- and below-ground plant components at the heavily and lightly grazed sites. Positive  $\delta^{15}\text{N}$  values indicate enrichment and negative  $\delta^{15}\text{N}$  values indicate depletion of  $^{15}\text{N}$  relative to atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N} = 0$ ). Mean values for 2011 and 2012 ( $\pm\text{SE}$ ) are presented and the asterisks denote significance between the heavily and lightly grazed sites at  $**p < .01$  and  $***p < .001$ . The deciduous shrub *Betula nana* was only present at the lightly grazed sites for both years. The  $\delta^{15}\text{N}$  for reindeer urine is about  $-2\text{‰}$



**TABLE 2** Analyses of variance of the within plant N pools differences and grazing intensity effects on plant  $\delta^{15}\text{N}$  natural abundance. Grazing intensity corresponds to the lightly and heavily reindeer grazed sites. The ecosystem N pools were *Betula nana*, Other deciduous shrubs, *Empetrum hermaphroditum*, Other evergreen shrubs, Forbs, Graminoids, Cryptogams, Litter and Roots

	df	$^{15}\text{N}$ natural abundance
Grazing intensity	1, 155	74.08***
Plant N pools	8, 155	19.70***
Grazing intensity: Plant N pools	7, 155	6.45***

F-values are presented along with the degrees of freedom (df) and the asterisks denote significance at \*\*\* $p < .001$ .

## 4 | DISCUSSION

Herbivores stimulate nutrient cycling in natural ecosystems by the conversion of plant biomass into dung and urine (Bardgett & Wardle, 2003), which has been suggested to not only increase nutrient availability for plants directly but also stimulate microbial activities and N mineralisation in the soil (Bardgett et al., 1998; Frank & Evans, 1997). Dung and urine provide approximately similar amount of N to ecosystems but the fraction excreted as urine increase dramatically with food quality (Hobbs, 1996). In addition, while dung is composed of a mixture of labile and non-labile N forms (Floate, 1970; Ruess & McNaughton, 1987; Seagle et al., 1992), most N in urine is in the form of urea that is easily taken up by plants directly or hydrolysed into ammonium (Witte, 2011). There is thus a great importance of understanding the function of urine in stimulating plant production and microbial activities and how nutrients from urine are incorporated in the N cycle in natural ecosystems.

Our  $^{15}\text{N}$  tracer study reveals that herbivore urine can be an important N source for plants in N-limited ecosystem, since most N from urea was rapidly incorporated in the plant canopy with only a small amount immobilized in soil microbial biomass or stored in plant roots. This study demonstrates that N from urine is utilized much faster than N added through dung (Barthelemy et al., 2015; van der Wal et al., 2004) or plant litter (Blok, Elberling, & Michelsen, 2016; Olofsson & Oksanen, 2002), in which nutrients are immobilized in the microbial biomass and it therefore can take years before nutrients are available to plants. Our data thus support a key role of urine in supporting a higher primary production and a fast pathway of nutrient return into the system. In the heavily grazed sites, approximately  $0.3 \text{ N g m}^{-2} \text{ year}^{-1}$  is deposited annually as urine, which corresponds to almost twice the atmospheric N deposition in the region, or 5%–10% of the N pool in photosynthetically active parts of plants. In the lightly grazed sites, where urine deposition is less than  $0.01 \text{ N g m}^{-2} \text{ year}^{-1}$ , urine surely may not support an overall higher plant production. However, urine is not distributed evenly on the soil surface and since all plants could efficiently take up N from the urea, urine can still be important for plant nutrition at a patch level.

Cryptogams were the largest sink of N from urine but interestingly, in contrast to what we hypothesized, they were still the largest sink of N even in the heavily grazed area where their abundance is low. By possessing effective absorptive surfaces, cryptogams are known to be able to capture large proportion of mineral nutrients (Crittenden, 1989; Eckstein & Karlsson, 1999; Jónsdóttir et al., 1995; Weber & Van Cleve, 1981) and organic N (Ayres, van der Wal, Sommerkorn, & Bardgett, 2006; Dahlman, Persson, Palmqvist, & Nasholm, 2004; Krab, Cornelissen, Lang, & van Logtestijn, 2008). Bryophytes have a capacity to retain most of the absorbed N for several years (Street, Burns, & Woodin, 2015; Weber & Van Cleve, 1981). High abundance of bryophytes could thus decrease N cycling rates, since they are long-lived, decompose slower than most vascular plants (Lang et al., 2009) and have a considerable capacity of internal recycling of N (Eckstein, 2000; Eckstein & Karlsson, 1999).

The strong reduction in the moss layer by reindeer recorded at the heavily grazed, in line with what have been observed in several places in the arctic (van der Wal & Brooker, 2004; van der Wal, van Lieshout, & Loonen, 2001), resulted in lower N uptake in cryptogam N pool and indicates that reindeer has the potential to increase N cycling and the growth of vascular plants by enabling more incoming nutrients to enter the soil. However, even in sites with a low grazing intensity, the moss layer is regularly disturbed by severe rodent peaks that occur every 3–4 years (Olofsson, Tommervik, & Callaghan, 2012). During such an event most of the N immobilized in the cryptogams could be released and become available for other ecosystem compartments. Our study coincided with the largest lemming peak for decades in northern Scandinavia, which dramatically reduced moss biomass during the winter 2011–2012 (Hoset, Kyro, Oksanen, Oksanen, & Olofsson, 2014; Olofsson et al., 2012). This probably explained the decrease in  $^{15}\text{N}$  recovery in the cryptogams already 1 year after the tracer addition since  $^{15}\text{N}$  enrichment did not decrease. This lemming peak also likely explains the substantial increase in litter between 2011 and 2012.

A similar capacity of plants to take up N from urine at both heavily and lightly grazed sites provides novel insights into the role of urine in ecosystems. Different taxa of vascular plants sequestered N from urine in proportion to their abundance in the vegetation; dwarf shrubs were just as efficient as graminoids and forbs. This indicates that urine deposition will support plant growth independent of vegetation composition. Thus, conclusions based on labile N injections into the soil showing clearly stratified N uptake among plant functional groups (Oulehlea, Rowea, Myšac, Chumanb, & Evans, 2016) may not be applicable to how plants take up N from urine deposition. Furthermore, our data show that urine addition does not automatically support a higher production of suitable food plants for the herbivores, since large amounts of N from urine were recovered in plants that are not preferred food for reindeer such as evergreen shrubs and bryophytes (Mathiesen, Aagnes Utsi, & Wenche, 1999; Thomas & Kroeger, 1981).

Although constituting a considerable ecosystem N pool, soil microbes sequestered only a marginal proportion of the N added in the form of urea irrespective of grazing intensity. This is in contrast to large

microbial immobilization of various N sources that have been injected into the soil (Clemmensen et al., 2008; Grogan, Michelsen, Ambus, & Jonasson, 2004; Jonasson et al., 1996; Nordin et al., 2004; Schimel & Chapin, 1996). The way N is entering the system may have high importance for plant N uptake. In studies where nutrients are applied over the plant canopy, which is a more realistic simulation of urine deposition by herbivores, mosses often capture most incoming nutrients and microbes often receive only a minor fraction (Jónsdóttir et al., 1995; Street et al., 2015; Weber & Van Cleve, 1981). Low sequestration of the added  $^{15}\text{N}$  by soil microbes is also consistent with earlier studies in the same site showing no effect on microbial N in response to experimental N addition and increased soil mineral N during an intensive grazing phase during reindeer migration (Stark & Väisänen, 2014). The  $^{15}\text{N}$  recovery was measured first after 2 weeks. The peak in microbial N immobilization could have appeared earlier than that, but this is still unlikely to explain the low  $^{15}\text{N}$  recovery in microbial biomass and high  $^{15}\text{N}$  recovery in plants in comparison to other studies (Clemmensen et al., 2008; Grogan et al., 2004).

A large fraction of the N from urea was recovered in the above-ground parts of vascular plants already after 2 weeks, which could reflect either a rapid mineralization of urea or a rapid absorption by the plant canopy. The capacity of arctic shrubs to absorb urea through their leaves has been demonstrated recently (Blok et al., 2016) and high foliar absorption rates of urea have been observed for many plant species in other biomes (Dong, Cheng, Scagel, & Fuchigami, 2002; Ruan & Gerendas, 2015; Uscola, Villar-Salvador, Oliet, & Warren, 2014). Canopy absorption of the  $^{15}\text{N}$ -urea should occur mainly through the cuticle which is permeable to soluble compounds (Baur, Buchholz, & Schonherr, 1997; Schreiber, 2005). Variations in cuticle permeability, leaf area, plant nutrient status, the presence of trichomes and in location and density of stomata have been presented to explain differences observed in urea absorption rates (Schreiber, 2005; Uscola et al., 2014). However, in our study, despite fundamental differences in plant traits and morphology, all plant functional groups seemed to be able to sequester N from urine.

Heavily grazed shrubs were less enriched by the labelled urea than the lightly grazed shrubs. This could indicate either a reduced capacity to take up the urea by the grazed shrubs, or a lower affinity to take up urea when mineral N concentrations in the soil are high (Stark & Väisänen, 2014). We suggest that a weaker capacity for N uptake from urea could constitute one mechanism contributing to the decrease in shrubs in grazed conditions. Thus, shrubs not only suffer more from the grazing damage but they are also less able to benefit from the increased nutrient availability than graminoids (McKendrick, Batzli, Everett, & Swanson, 1980). Differences in plant nutrient acquisition and resorption strategies can explain the higher  $^{15}\text{N}$  retention in roots at the heavily grazed sites, as the abundant graminoids have a high N uptake rate and transfer more of their nutrients into their roots than shrubs (Aerts, 1999; Aerts & Chapin, 2000).

The litter layer was also a fairly large sink of N from urea. While parts of the  $^{15}\text{N}$  enrichment in the litter in the second year could derive from senesced plant materials that previously sequestered the  $^{15}\text{N}$ , this would only correspond to a small fraction of the  $^{15}\text{N}$

enrichment at 2 weeks since the plant biomass was harvested at the peak of the growing season, long before plant senescence. These results rather indicate a considerable  $^{15}\text{N}$  immobilization by the microbial communities living in the litter layer (Ewing, Groffman, & Frank, 2010; Zaady, Groffman, & Shachak, 1996). Although we did not directly measure this process, this interpretation is supported by previous studies demonstrating the high biological activity in this layer with the conversion of inorganic N from precipitation into organic forms in the litter (Seastedt, 1985). This thin boundary between the above- and below-ground compartments of the ecosystem seems to have a crucial role in the N cycling in tundra ecosystems by immobilizing a large amount of the incoming easy available N. However, this compartment does not constitute a long-term sink for the incoming N since the  $^{15}\text{N}$  enrichment was largely reduced after 1 year and N pools in the litter were small compared to those in the soil and vegetation (Olofsson et al., 2004). Our findings thus support that early phases of organic matter decomposition in the fresh plant litter are likely to be limited by N availability, whereas the later phases of decomposition in the soil organic matter could be limited by labile C availability. By this way, the urine added to the litter layer in the heavily grazed area could contribute to the generally faster litter decomposition rates in grazed sites (Olofsson & Oksanen, 2002).

The small variation in plant  $^{15}\text{N}$  natural abundance among taxa in heavily grazed sites supports previous findings that plants are using more similar N sources in the heavily grazed sites than under lightly grazed conditions (Barthelemy, Stark, Kytöviita, & Olofsson, 2017; Barthelemy, Stark, Michelsen, & Olofsson, 2017). Since a substantial amount of N is added to the system in the form of urine,  $^{15}\text{N}$ -urea recovery was high for all the plants at the site and plant  $^{15}\text{N}$  signatures converged towards the expected  $\delta^{15}\text{N}$  values from dung and urine ( $-2$ ) (Barthelemy, Stark, Kytöviita, et al., 2017; Barthelemy, Stark, Michelsen, et al., 2017; Finstad & Kielland, 2011), urine uptake of all taxa could be the main driving mechanism for the reduced variation in  $\delta^{15}\text{N}$  values in heavily grazed conditions. Our data thus provide further support that urine is an important nutrient source for arctic plants.

Our study demonstrated a rapid sequestration of the applied  $^{15}\text{N}$ -urea by plants, indicating that arctic ecosystems are well adapted to take advantage of these N pulses. However, the total tracer recovery was lower in the heavily grazed than in the lightly grazed tundra, indicating that this more nutrient-rich condition might lead to a more open N cycle and higher losses. Both the higher nutrient cycling, the sharp decline of nutrient-conservative plant species and a reduced N transfer from mycorrhiza fungi could have contributed to a lower N retention at the heavily grazed tundra (Aerts & Chapin, 2000; Templer et al., 2012). There are several pass-ways through which the applied N could be lost, including  $\text{NH}_3$  volatilization (Bussink & Oenema, 1998) and leaching (Treat, Wollheim, Varner, & Bowden, 2016). However, given the small plot sizes, losses can be overestimated due to N uptake by plants outside the treatment plots. Litter reallocation and reindeer consumption of biomass inside the plots should be also included in the

losses. Interestingly, the total N pools did not differ between the two grazing systems. Further investigations are needed to understand how high N availability and unchanged N pools can be maintained after 50 years of grazing given the lower N retention at the heavily grazed sites, but this could reflect a constant N input from reindeer through depositing more N in the waste products than what is removed through consumption of plants.

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## AUTHORS' CONTRIBUTIONS

H.B. and J.O. conceived the ideas and designed methodology; H.B. performed the sample collection, prepared all samples and conducted statistical analysis and led writing of the manuscript; H.B. and S.S. conducted soil analysis; A.M. contributed to the nitrogen isotope analysis. All authors contributed critically to the drafts and gave final approval for publication.

## DATA ACCESSIBILITY

Data associated with this article are archived at the Dryad Digital Repository: <https://doi.org/10.5061/dryad.52qh9> (Barthelemy, Stark, Michelsen, et al., 2017).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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